Atherosclerosis 273 (2018) 37–44

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

# Effects of gastric bypass surgery followed by supervised physical training on inflammation and endothelial function: A randomized controlled trial

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ARTICLE INFO

*Article history:*
Received 30 January 2018
Received in revised form
13 March 2018
Accepted 5 April 2018
Available online 6 April 2018

*Keywords:*
C-reactive protein (CRP)
Exercise
Intercellular adhesion molecule 1 (ICAM-1)
Interleukin 6 (IL-6)
Tissue-type plasminogen activator antigen (t-PA:Ag)
Von willebrand factor (vWF)

ABSTRACT

*Background and aims:* Obesity and physical inactivity are both associated with low-grade inflammation and endothelial dysfunction. Bariatric surgery improves markers of inflammation and endothelial function, but it is unknown if physical training after bariatric surgery can improve these markers even further. Therefore, we aimed to investigate the effects of Roux-en-Y gastric bypass (RYGB) followed by physical training on markers of low-grade inflammation and endothelial function.
*Methods:* Sixty patients approved for RYGB underwent examinations pre-surgery, 6, 12, and 24 months post-surgery. Six months post-surgery, they were randomized 1:1 to an intervention group or a control group. The interventions consisted of two weekly sessions of supervised moderate intensity physical training for a period of 26 weeks. Fasting blood samples were analyzed for concentrations of interleukin 6 (IL-6), C-reactive protein (CRP), intercellular adhesion molecule 1 (ICAM-1), tissue-type plasminogen activator antigen (t-PA:Ag) and von Willebrand factor (vWF).
*Results:* RYGB markedly improved markers of inflammation (IL-6, CRP) ($p < 0.001$) and endothelial function (ICAM-1, t-PA:Ag, vWF) ($p < 0.05$), and the improvements were sustained 24 months post-surgery ($p < 0.01$), except for the effects on vWF. We found no correlations between the changes in weight or BMI and the changes in markers of inflammation and endothelial function, except that the change in vWF was found to be inversely correlated with the changes in weight and BMI. We observed no effects of supervised physical training on markers on inflammation or endothelial function ($p>0.1$ for all).
*Conclusions:* RYGB causes substantial and sustained favorable effects on markers of inflammation and endothelial function. Supervised physical training after RYGB did not cause additional improvements.


## 1. Introduction

The prevalence of obesity increases worldwide, and obesity is associated with increased morbidity and mortality [1,2]. The leading cause of morbidity and mortality related to high BMI is cardiovascular disease (CVD) [1].

Obesity is a condition with accumulation of excessive body fat in white adipose tissue (WAT). WAT is an active endocrine organ which synthesizes and secretes cytokines, including the pro-inflammatory mediator Interleukin 6 (IL-6) [3]. These cytokines promote an inflammatory state in vascular cells and stimulate the expression of adhesion molecules, e.g. intercellular adhesion molecule 1 (ICAM-1), thereby inducing endothelial dysfunction with increased levels of von Willebrand factor (vWF) and tissue-type plasminogen activator antigen (t-PA:Ag). In the liver, the cytokines stimulate the hepatocytes to produce acute phase proteins, e.g. C-reactive protein (CRP) [4]. The chronic inflammatory state in obesity contributes to the disease processes linked with obesity,

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https://doi.org/10.1016/j.atherosclerosis.2018.04.002
0021-9150/

such as type 2 diabetes and CVD [5].

Bariatric surgery has pronounced effects on CVD mortality and morbidity [6]. A possible explanation could be that bariatric surgery reduces concentrations of CRP [7–19], IL-6 [7–10], ICAM-1 [18–20], t-PA:Ag [21–23], and vWF [10–12], which markedly improves the low-grade inflammation and endothelial dysfunction in people with obesity. Inflammation and endothelial dysfunction contribute to atherosclerosis, and biomarkers of systemic inflammation and endothelial function have been shown to be predictive for future CVD [24,25].

Bariatric surgery candidates are physically inactive compared to normal weight individuals [26]. It has not been fully elucidated if bariatric surgery alone motivates patients to be more physically active [27,28], or whether it is also necessary to promote physical activity with exercise interventions. Physical inactivity is independently associated with low-grade inflammation, endothelial dysfunction and increased risk of CVD [29,30]. It is well-documented that regular exercise reduces the risk of CVD [31], however, the results regarding effects of moderate intensity exercise on low-grade inflammation and endothelial function are inconsistent [32–40].

To date, no randomized controlled trials have investigated the effect of physical training after Roux-en-Y gastric bypass (RYGB) on biomarkers of inflammation and endothelial function. Therefore, the aim of this study was to evaluate the effect of a supervised physical training intervention following RYGB on biomarkers of inflammation and endothelial function. Furthermore, this study provides information about the effect of RYGB on the same biomarkers as both pre- and post-surgery measurements before initiation of the physical training were performed.

## 2. Materials and methods

### 2.1. Participants and study design

The current paper is part of a study investigating the effects of supervised physical training following RYGB on weight loss, physical activity, health related quality of life, and markers of CVD. Results reported in this paper are based on secondary outcome variables. The study design has been described in detail elsewhere [41]. The study was conducted according to the declaration of Helsinki, approved by the local Ethics Committee (Project-ID: S-20120112), and the trial was registered at http:/www.ClinicalTrials.gov (No. NCT01690728).

In brief, we included 60 non-smoking participants, eligible for RYGB according to the national guidelines (BMI > 35 kg/m$^2$ with obesity related disease or BMI > 50 kg/m$^2$ with obesity related social or physical complications). All subjects gave oral and written informed consent. The patients underwent laparoscopic RYGB at the Hospital of Southwest Jutland. The surgery was performed by one out of a group of three skilled surgeons, with a 20–30 mL gastric pouch, a 60 cm bilio-pancreatic limb and a Roux limb of 150 cm. Six months after RYGB the participants were randomized 1:1 to either a supervised physical training intervention group (INT, n = 32) or a control group (CON, n = 28). Randomization was done by one of the two principal investigators (CRS or LHM), by the sealed envelope method and performed in blocks of four, ensuring an equal distribution of type 2 diabetes patients. We excluded patients using vitamin K antagonists or hormones. Physically disabled patients and patients with severe osteoarthritis were also excluded. Due to co-morbidities some of the participants received various types of medicine, e.g. metformin (n = 16), liraglutide (n = 4), ACE inhibitors (n = 21), and statins (n = 10). Nineteen patients did not receive any medical treatment.

The intervention consisted of two weekly sessions of 40 min of supervised physical training for 26 consecutive weeks at a local fitness center. The participants in INT were provided with free access to the fitness center during the intervention period. The training sessions combined moderate intensity endurance and resistance training and were supervised by physiotherapists. The supervising physiotherapist ensured weekly progression in resistance and endurance training. Besides the supervised physical training, the participants in INT were encouraged to be physically active with a goal of a total of 210 min per week, corresponding to the guidelines from the Danish National Board of Health. CON were given the clinic's standard information about the importance of being physically active after RYGB. The dietary recommendations were similar in both groups, corresponding to a normal post-bariatric dietary counselling, securing sufficient protein and vitamin intake. The flow of participants in the study is presented in Fig. 1. Among participants in INT, 19 were compliant to the intervention (attending > 50% of the supervised physical training sessions) corresponding to 59.4% of all study participants allocated to INT.

The subject characteristics pre- and post-surgery as well pre- and post-intervention are presented in Table 1. These results were published previously [41] and showed that body weight and BMI improved markedly as a result of RYGB, and that supervised physical training resulted in a better weight maintenance 24 months post-surgery in INT compared to CON.

### 2.2. Blood sampling

Blood samples were collected between 7.45 and 8.30 in the morning after 10 h of fasting. Venous blood samples were collected with minimal stasis after 15 min rest in a supine position. The first 5 mL collected were discarded. The following 4 mL were collected in clot activator tubes (Becton-Dickinson, Plymouth, UK; BD Ref: 369032) and used for analyses of insulin and CRP in serum. Next, 3 mL were collected in trisodium citrate tubes (0.109 mol/L $Na_3$Citrate, BD Ref: 363048) for analysis of vWF and t-PA:Ag. Finally, 3 mL were collected in EDTA-tubes ($K_2$-EDTA: 5.4 mg, BD Ref: 367525) for IL-6 and ICAM-1, and 3 mL were collected in EDTA-tubes with citric acid and sodium fluoride (Vacuette FC Mix Tube, Greiner Bio-One, Frickenhausen, Germany, Ref: 454513) for plasma glucose measurements. Immediately after sampling, platelet poor plasma was prepared by centrifugation for 20 min at 2000 × *g* (20 °C). Plasma and serum were transferred to aliquots, rapidly frozen and stored at −80 °C until testing.

### 2.3. Blood analyses

Plasma glucose (mmol/L) was analyzed immediately with an Architect C16000 (Abbott Diagnostics Division, Copenhagen, Denmark). Plasma and serum samples were thawed in a water bath at 37 °C and analyzed in one series for each individual. Insulin (pmol/L) was measured using a commercial electrochemiluminescence immunoassay (COBAS, Roche Diagnostics, Germany). Concentrations of CRP (mg/L) were determined on a nephelometer (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). Commercially available ELISA kits were used for the measurements of plasma levels of ICAM-1 (ng/mL)(Allele-specific Quantikine; R&D Systems, Oxon, UK) and IL-6 (pg/mL) (Quantikine High Sensitivity; R&D Systems). Concentrations of vWF (%) were determined by an in-house ELISA using rabbit anti-human vWF polyclonal IgG as capture and detecting antibodies (DAKO, Glostrup, Denmark, Ref. Nr. A0082). Concentrations of t-PA:Ag (ng/mL) were determined by an in-house ELISA using mouse anti-human t-PA monoclonal IgG as capture (clone 15-4-21) and detection (clone 15-4-6) antibodies.